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"Cytomegalovirus Disease" in Renal Allograft Recipients: Is Human Herpesvirus 7 a Co-Factor for Disease Progression?

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Fifty-six renal allograft recipients were studied prospectively for 3 months or longer after transplant. The polymerase chain reaction (PCR) was used to screen peripheral blood leucocyte (PBL) specimens for CMV, human herpesvirus 6 (HHV6) and human herpesvirus 7 (HHV7) DNA (DNAemia) in 67 healthy controls and in serial (fortnightly) PBL specimens from the 56 allograft recipients. None of the healthy controls had detectable CMV DNAemia, although HHV6 and HHV7 DNAemia was found in 7% and 9% of individuals respectively. In contrast, DNAemia due to CMV, HHV6 and HHV7 was found in 50%, 36% and 39% of patients respectively, at some time during the post-transplant period. Of the 28 patients who had CMV DNAemia, eight developed "CMV disease." The risk of progression to "CMV disease" was increased in patients with concurrent DNAemia to all three viruses (relative risk 3.7; 95% CI 1.3–10.5). The relative risk of "CMV disease" for patients with concurrent CMV and HHV7 was also increased (RR = 3.5; 95% CI = 1.1–11.6), while the association between CMV and HHV6 was inconclusive (RR = 2.1; 95% CI = 0.7–6.6). The first 26 patients recruited to the study also had serial serum samples tested for antibody responses to the three viruses. "CMV disease" was associated with rising antibody titres to HHV7 (Fisher's exact test, $P = 0.02$), and weakly so with HHV6 ($P = 0.07$). It is concluded that in patients with CMV DNAemia, concurrent infection/reactivation of HHV7 (and possibly HHV6) is associated with an increased risk of progression to "CMV disease."

KEY WORDS: human herpesvirus 6, renal transplantation, PCR, serology, viral co-factor

INTRODUCTION

Two lymphotrophic human herpesviruses, human herpesvirus-6 (HHV6) and human herpesvirus-7 (HHV7), have been discovered during the last 9 years [Salahuddin et al., 1986; Frenkel et al., 1990]. The two viruses are distinct from each other and from other known human herpesviruses [Berneman et al., 1992], but both have close DNA homology to cytomegalovirus and are likely to be classified as β-herpesviruses [Thomson et al., 1991; Black and Pellet, 1993].

HHV6 and HHV7 infect the CD4-T lymphocyte [Thomson et al., 1991; Black and Pellet, 1993]. Antibodies to both viruses can be detected in approximately 90–95% of the adult population, much of the infection occurring in early childhood [Clark et al., 1993]. They cause the common childhood febrile illness exanthem subitum [Yamanishi et al., 1988; Tanaka et al., 1994].

Infections with human herpesviruses may lead to serious complications in solid organ transplant recipients and cytomegalovirus (CMV), in particular, is associated with significant morbidity and mortality. Although cytomegalovirus infection or reactivation is common in the post-transplant phase, only some of these patients develop symptomatic disease. While the degree of immunosuppression and donor seropositivity are associated with increased risk of progression to "CMV disease," it is not clear that the pathogenesis of "CMV disease" is explained exclusively by these two factors. There have been preliminary reports that infections with HHV6 cause illness in transplant recipients similar to that caused by CMV [reviewed by Lusso and Gallo, 1994; Cone et al., 1994], but establishing conclusively a pathogenic role for an ubiquitous and persistent virus has proved difficult.

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HHV6 has been isolated from donor kidneys at the time of transplantation, and could potentially be transmitted to the organ recipient by this means [Yoshikawa et al., 1992]. There has been only one report on HHV7 activity in immunocompromised patients to date [Yalcin et al., 1994].

We studied renal allograft recipients to investigate disease association with HHV6 and HHV7 infections and to investigate interactions, if any, between these two viruses and CMV.

METHODS

Patients and Specimens

Fifty-six patients (median age 47 years; range 4–70 years), 34 males and 22 females, receiving renal allografts at the Royal Victoria Infirmary during 1991–92 were studied prospectively. Post-transplant immunosuppression was based on cyclosporin A, with prednisolone and azathioprine being added depending on the history of previous immunological graft loss or degree of human leucocyte antigen (HLA) A-B and DR mismatches. The first two episodes of clinically diagnosed graft rejection were treated with intravenous steroids. Failure to respond to therapy or a third episode of rejection led to renal biopsy. Anti-thymocyte globulin was used if there was evidence of vascular or severe cellular rejection, or a failure to respond to steroids.

Urine, heparinised (lithium sodium 100 units/10 ml blood) and clotted blood specimens were collected pretransplant and at 14 day intervals for 12–30 weeks post-transplant (mean duration of follow up 23 weeks). In the event of clinically suspected CMV disease, other appropriate specimens (throat swab, broncho-alveolar lavage, intestinal biopsy) were also tested in addition to those listed above.

Specimens of heparinised and clotted blood were also obtained after informed consent from 68 healthy adults being tested for immunity after hepatitis B immunisation to serve as a control population. None of these healthy adults was a laboratory worker.

Definitions of CMV Disease

In this paper, CMV disease is defined as one of the following [based on Ljungman and Griffiths, 1993]: 1) "CMV syndrome"—pyrexial illness with a leucopenia/fall in platelet count with or without a transaminitis for which no other cause could be found, combined with laboratory evidence of CMV infection (i.e., CMV IgM detection, rising CF antibodies, viruria or viraemia); 2) CMV pneumonitis—clinical evidence of pneumonitis (hypoxemia and/or radiographic changes) associated with the detection of CMV (DEAFF test, viral culture or direct antigen detection) [Griffiths et al., 1984] in broncho-alveolar lavage, lung biopsy or post-mortem tissue, and in the absence of another cause; 3) CMV gastrointestinal disease—gastrointestinal symptoms together with histological evidence of CMV in a biopsy of the lesion. Culture of CMV from the biopsy tissue in the absence of histological evidence of "CMV disease" was regarded as presumptive CMV gastrointestinal disease.

The results of HHV6, HHV7 and CMV PCR assays were available only in retrospect, and influenced neither the diagnosis nor the management of CMV disease during the course of this study.

Virology

Polymerase chain reaction (PCR). Peripheral blood leucocytes (PBL) were separated from heparinised blood specimens by buoyant density centrifugation on Histopaque 1119 (Sigma). Any contaminating red blood cells were removed by osmotic lysis, the PBL washed in phosphate buffered saline, 2.5×10^6 cells resuspended in 0.5 ml of a PCR-compatible proteinase K buffer (containing 0.45% Nonidet P40 and 0.45% Tween 20 rather than sodium lauryl sulphate) [Higuchi, 1989] and stored frozen at −40°C until tested.

The PBL suspensions were digested with proteinase K at 55°C for 1 hour, the enzyme inactivated by heating at 95°C for 10 minutes, and 5 μl of the supernatant used for PCR amplification (total reaction volume 50 μl). One round CMV and nested HHV6 PCRs were performed using the primers and conditions described previously [Demmler et al., 1988; Peiris et al., 1995; Wakefield et al., 1992].

HHV7 PCR was carried out using the primers H7 and H8 described by Berneman et al. [1992]. The agarose gel electrophoresis results were confirmed by dot-blot hybridization using a digoxigenin-labelled oligonucleotide probe detected by a chemiluminescent reaction (Boehringer-Mannheim) according to the manufacturer's instructions.

Every fourth specimen was followed by a negative control (proteinase K digested cell lysis buffer). Preparations of CMV (strain AD169), HHV6 (AJ strain) [Tedder et al., 1987] and HHV7 (DC strain (D. Clark, R.F. Jarrett, unpublished results) viruses grown in human embryo fibroblast cells, JJHAN (or HSB-2) and Sup-T1 cells respectively were digested with proteinase K as above, and aliquots at limiting dilution for the PCR assay (i.e., 10^{-3}–10^{-6}) were used as positive controls throughout the study. The precautions detailed by Kwok and Higuchi [1989] to avoid PCR cross-contamination were adhered to. If any of the negative controls gave a positive reaction, the whole PCR run was repeated. All the PBL extracts were also tested for β-globin DNA using PCR [Lo et al., 1989] to confirm the presence of PCR amplifiable DNA, and to exclude presence of PCR inhibitors.

Serology. Sera were tested for HHV6 and HHV7 antibodies by an indirect immunofluorescence test as described previously [Salahuddin et al., 1986]. Briefly, JJHAN cells infected with the AJ strain of HHV6 and Sup-T1 cells infected with the DC strain of HHV7 were spotted onto 12-well teflon-coated glass slides (Hendley-Essex, UK). The slides were air dried, fixed with cold acetone and kept dry at −20°C until used. Slides prepared similarly from uninfected JJHAN and Sup-T1 cells were used as controls. Sera were diluted in PBS in doubling dilutions starting from 1/50. Each dilution was put onto a test and control well and the slides were incubated for 30 minutes at 37°C in a moist chamber. After washing

three times with PBS, each for 5 minutes, fluorescein isothiocyanate–labelled goat antihuman IgG antibody (Dako Ltd) was put in each well. Following incubation for another 30 minutes at 37°C, the slides were washed three times with PBS as before and once with distilled water. They were then examined at a magnification of 1500× using a fluorescence microscope (Olympus) with epi-illumination and interference filters. Antibody titres were expressed as the reciprocal of the highest serum dilution yielding detectable specific fluorescence.

Sera were tested for CMV IgM by ELISA (Captia CMV-M, Mercia Diagnostics). If there was clinical suspicion of CMV disease, paired sera were tested by complement fixation (CF) to document rising antibody titres.

Surveillance cultures for CMV. Urine and leucocytes (2×10^5) separated as above were tested for CMV by the DEAFF test [Griffiths et al., 1984] using the monoclonal antibody 6A2 to the CMV major immediate early (MIE) protein (kindly provided by Dr. Andrew Fox, Manchester), and the reaction visualised by immunofluorescence or immunoperoxidase techniques.

Other virological tests. When there was clinical suspicion of infection, appropriate clinical specimens were cultured on human embryo fibroblast, HEp-2 and rhesus monkey kidney cells. Cells from respiratory specimens (sputum, broncho-alveolar lavage or lung biopsy) were stained by immunofluorescence to look directly for antigens of influenza viruses A and B, respiratory syncytial virus, adenovirus, parainfluenza viruses types 1–4, herpes simplex virus, varicella zoster virus and measles viruses [Gardner and McQuillin, 1980].

Statistical Analysis

Fisher's exact test was used to test associations. Relative risks and confidence interval analysis were carried out using the Epi-info version 6 software package.

RESULTS

Seroprevalence prior to transplant for CMV, HHV6 and HHV7 viruses was 68%, 95% and 98% respectively. Both organ donor and allograft recipient were seronegative for CMV in six cases. In none of the transplants were both donor and recipient seronegative to either HHV6 or HHV7. Twelve CMV seronegative allograft recipients received organs from a seropositive donor and were given oral acyclovir prophylactically at a dose of 800 mg 8 hourly (dose adjusted if required for renal function) for 3 months post-transplant.

Virus DNAemia

The PCR primers used to detect CMV, HHV6 and HHV7 DNA respectively did not cross amplify DNA from the others, or from other human herpesviruses (herpes simplex types 1 and 2, varicella-zoster virus, Epstein-Barr virus), or from adenoviruses (which are known to have some DNA homology with CMV). The amplified product of expected size was detected by agarose gel electrophoresis in all three PCRs. For CMV and HHV7, the specificity of the band on agarose gels was confirmed by dot blot oligonucleotide hybridization. The inner primers of the nested HHV6 PCR served to confirm the specificity of the product amplified in the first round.

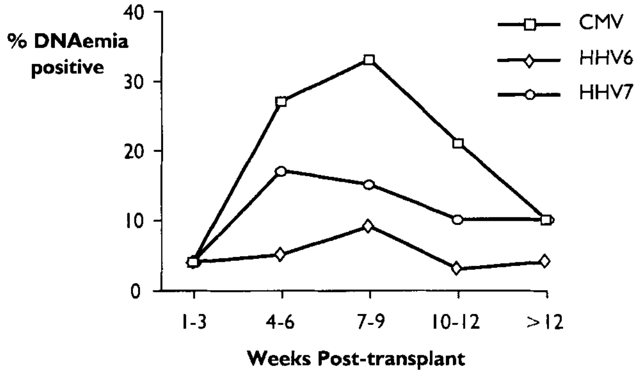

Fig. 1. Proportion of PBL specimens positive for CMV, HHV6 and HHV7 virus DNA at different times after renal transplant.

The sensitivity of the PCR reactions for CMV and HHV6 DNA was estimated by using cloned virus DNA containing a defined copy number of the genome. The CMV PCR detected approximately 100 copies of viral DNA in a 5 μl volume (containing 2.5×10^4 PBL), while the HHV6 PCR detected 100–200 copies per 5 μl for the first round PCR. The sensitivity of the PCR for HHV7 (PCR, followed by dot-blot hybridisation with a digoxigenin labelled oligonucleotide probe) was not estimated in terms of copies of genome detected, but the HHV7 assay was positive to a dilution of 10^{-6} of an extract of virus-infected Sup-T1 cells. HHV6 DNA extracted from infected JJhan cells and detected by the HHV6 PCR gave a similar end-point.

None of the 68 healthy controls (38 of whom were CMV seropositive) was positive for CMV DNA by PCR in PBL extracts (DNAemia). However, five (7%) and six (9%) of healthy control individuals were positive for HHV6 and HHV7 DNAemia respectively. In contrast 50%, 36% and 39% of the 56 renal allograft patients were DNAemia positive for CMV, HHV6 and HHV7 viruses, respectively, on at least one occasion after transplantation.

Figure 1 shows the proportion of specimens positive for each of the viruses at different times post-transplant. CMV DNAemia is maximal at 7–9 weeks post-transplant whereas HHV7 DNAemia peaks earlier (4–6 weeks post-transplant). With HHV6 DNAemia, however, there is no obvious pattern in relation to time post-transplant. In addition, HHV6 DNAemia is usually sporadic, with a single positive specimen being preceded and followed by negative specimens (Fig. 2, patients B,D,E). In contrast, when a patient became CMV or HHV7 DNAemia positive, there followed a number of consecutive positive samples before reverting to negativity (Fig. 2, patients A,C,D). Of patients becoming DNAemia positive for CMV and HHV7 respectively, 46% and 45% had three or more consecutive positive samples, in contrast to 10% for HHV6. No obvious clinical syndromes could be attributed to patients becoming DNAemia positive by either

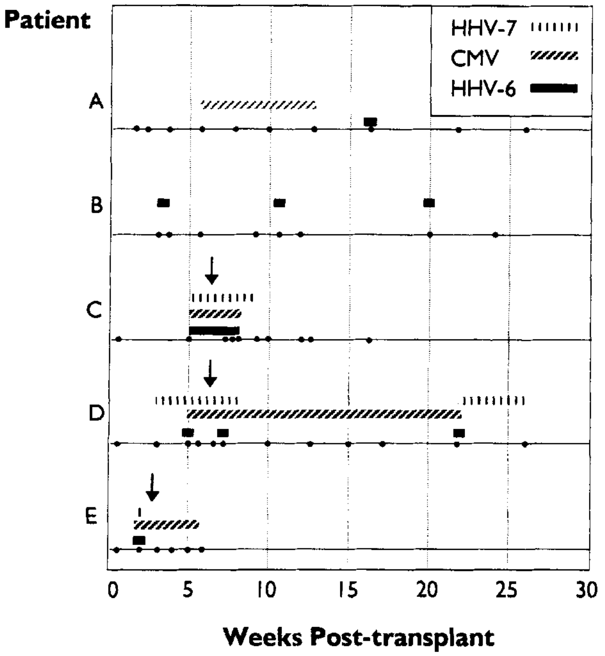

Fig. 2. Profile of virus DNAemia in five patients after transplant. ● denotes the time of collection of the PBL samples. Consecutive positive samples are denoted with solid or hatched bars. ↓ denotes the time of onset of clinical symptoms (if any).

HHV6 or HHV7 virus alone. However, since the HHV6 and HHV7 PCR results were available only in retrospect, subtle clinical changes associated with DNAemia by either virus could not be excluded.

Twenty-eight of the 56 patients studied became CMV DNAemic post-transplant, eight of whom were recorded as having "CMV disease" (pneumonitis 2; gut infection 2; pyrexial syndrome 4). All eight patients with disease had CMV DNAemia at the time of onset of clinical symptoms. DNAemia profiles of three patients (C,D,E) with "CMV disease" are shown in Figure 2, with the arrow denoting the onset of clinical symptoms. Patient D was an interesting example. He was CMV and HHV7 seronegative, and HHV6 seropositive pre-transplant. The donor was seropositive to all three viruses, and acyclovir (800 mg 8 hourly orally) was used post-transplant. He became HHV7 DNAemic on day 19 post-transplant, and DNAemic for both CMV and HHV6 at day 33 post-transplant. He had a pyrexial illness with abnormal liver function and thrombocytopenia on day 44 post-transplant, developed a CMV IgM response and was started on ganciclovir (10 mg/kg/day iv in two divided doses for 14 days) on the next day. He responded clinically but remained positive for CMV DNAemia although the DNAemia titre [see Peiris et al., 1995] declined from 100 to 10. However the HHV7 DNAemia ceased as he improved clinically. Sera collected between days 33 and 50 post-transplant showed seroconversion to HHV7, and a rise in antibody titre (50 to 200) to HHV6.

The risk of patients with CMV DNAemia developing clinically overt "CMV disease" was 8/28 (29%). A number of the patients with "CMV disease" (e.g., patients C,D,E in Fig. 2) also had evidence of HHV7 and/or HHV6 DNAemia. In the 28 patients with CMV DNAemia, the association between concurrent HHV7 or HHV6 DNAemia and progression to disease within the first 90 days after transplant was studied. Concurrent DNAemia was defined as CMV DNAemia combined with either HHV6 or HHV7 DNAemia in the same or consecutive specimens, less than 7 days apart. Concurrent DNAemia was considered to be related to "CMV disease" if found within 1 week of the onset of symptoms, and one patient found positive only 7 weeks later was not regarded as "concurrent DNAemia" for the purpose of this analysis. Of the eight patients with "CMV disease," five and four had evidence of concurrent HHV7 and HHV6 respectively (Table I). In the 28 patients with CMV DNAemia, the relative risk of developing symptomatic disease was increased 3.7-fold in patients with concurrent HHV7 and HHV6 DNAemia (95% CI 1.3–10.54) (Table I), and increased 3.5-fold (95% CI 1.1–11.6) in patients with concurrent CMV and HHV7. The relative risk of disease in patients with concurrent HHV6 and CMV DNAemia increased by 2.1-fold (95% CI 0.7–6.6).

Thirty-two of the 56 patients studied who had clinically diagnosed episodes of graft rejection were treated with intravenous methyl prednisolone and/or anti-thymocyte globulin. Patients receiving anti-rejection therapy were more frequently DNAemic with CMV (66% vs. 31%; Fisher's exact test, $P = 0.05$). Although anti-rejection therapy was associated with an increase in the frequency of HHV6 (47% vs. 25%; $P = 0.25$) and HHV7 (41% vs. 25%; $P = 0.46$) DNAemia, these differences were not statistically significant at the 95% level of confidence.

In order to control for independent associations between immunosuppressive anti-rejection therapy on HHV6 or HHV7 activity on the one hand, and on "CMV disease" on the other, we repeated the analysis on the subset of 21 patients receiving anti-rejection therapy (i.e. excluding patients not having antirejection therapy from the analysis). The relative risks for "CMV disease" in patients with CMV DNAemia concurrent with HHV6 and HHV7 (RR = 3.2; 95% CI 1.2–8.3); HHV7 (RR = 3.3; 95% CI 1.1–10.1); or HHV6 (RR = 1.6; 95% CI 0.6–4.7) were almost identical to those seen in the whole group of 28 patients.

Serological Responses to CMV, HHV6 and HHV7

The first 26 patients recruited to the study (including four patients with "CMV disease") had serial serum samples (taken prior to transplant, and at approximately 30, 60 and 90 days post-transplant) which were titrated for IgG antibodies to HHV6 and HHV7 IgG. The same sera were also tested for IgM antibodies to CMV. A detailed analysis of the serological responses and their correlation with DNAemia is reported elsewhere (manuscript in preparation), but 13 patients had CMV IgM responses, while eight and two respectively had rising titres to HHV6 and HHV7. Both patients with an antibody re-

TABLE I. Patients With CMV DNAemia (n = 28): Does Concurrent HHV6 or HHV7 DNAemia Increase the Risk of "CMV Disease?"

	"CMV disease" (n = 8)	No disease (n = 20)	Relative risk of disease (95% CI)[b]
Concurrent DNAemia[a] and			
CMV + HHV6 + HHV7	4/8 (50%)	2/20 (10%)	3.7 (1.3–10.5)
CMV + HHV7	5/8 (63%)	4/20 (20%)	3.5 (1.1–11.6)
CMV + HHV6	4/8 (50%)	5/20 (25%)	2.1 (0.7–6.6)

[a]Concurrent DNAemia was defined as two or more viruses detected by PCR in PBL within a 7 day interval.
[b]CI, confidence intervals.

TABLE II. Association Between Serological Responses to CMV, HHV6, HHV7 and "CMV Disease" in 26 Patients

	"CMV disease"	No disease	
CMV IgM response	3/4 (75%)	9/22 (41%)	$P = 0.31$[a]
HHV6 rise in titre	3/4 (75%)	5/22 (23%)	$P = 0.07$
HHV7 rise in titre	2/4 (50%)	0/22 (0%)	$P = 0.02$

[a]Fisher's exact test.

sponse to HHV7 also had rising titres to HHV6 and detectable CMV IgM.

Four of these 26 patients were diagnosed to have "CMV disease." Although the numbers are small, "CMV disease" was associated with rising antibody titres to HHV7 virus ($P = 0.02$), showed a weak association with HHV6 virus ($P = 0.07$), but showed no association with CMV IgM responses ($P = 0.31$) (Table II). The patients with "CMV disease" and rising titres to HHV6 and/or HHV7 had concurrent DNAemia with the respective virus(es).

DISCUSSION

The investigation of HHV6 and HHV7 viruses as human pathogens poses several problems. Virus culture which, with most other viruses, is reliable evidence of active viral replication, may not necessarily have the same significance with HHV6 and HHV7. The culture method used for HHV6 and HHV7 is co-cultivation of infected cells with a susceptible cell line. A positive culture may therefore reflect active virus replication at the time the specimen was collected or reactivation of virus which had been latent. Detection of HHV6 or HHV7 viral DNA in PBL specimens is similarly not evidence of active viral replication and may well reflect latent virus. With the PCR method we use to detect CMV DNAemia, our previous studies have shown that viral DNA is undetectable in PBL from healthy donors and that CMV DNAemia correlates empirically with active viral replication [Peiris et al., 1995]. With HHV6 and HHV7, however, virus DNA is detectable in a proportion (7–9%) of healthy individuals. Other studies have found a higher prevalence of HHV6 DNA in the PBL of healthy controls than we observed [Gopal et al., 1990; Di Luca et al., 1994]. These contrasting results may be due to differences in the sensitivity of the PCR methods used and the number of leucocytes sampled. Our analysis was based on results from a single round PCR using 2.5×10^4 cells/PCR tube.

Whether detection of HHV6 or HHV7 DNA in PBL specimens is an indication of persistent low level viral replication or of the presence of latent virus in PBL is unclear. In an individual patient, therefore, it is difficult to interpret the significance of either viral culture for HHV6 or HHV7, or DNAemia as detected by PCR. However, the overall pattern of HHV6 and HHV7 DNAemia in a cohort of patients may throw some light on the pattern of activity of these viruses, and this forms the justification for our study.

The number of patients who were DNAemia positive following renal transplantation was higher than in the healthy control population. Since repeated PBL specimens were tested from patients receiving renal transplants, this observation may be a sampling artefact. However, if specimens from the patient cohort as a whole at different times after transplantation are considered, there is an increase in the proportion positive for CMV and HHV7 DNAemia, but they peak at 7–9 weeks and 4–6 weeks respectively (Fig. 1). This observation is compatible with an increase in viral reactivation following post-allograft immunosuppression. With HHV6, however, there is no obvious change in the proportion of specimens that are DNAemia positive in relation to time post-transplant (Fig. 1), and this is not different to that seen in the control population.

When DNAemia occurs, CMV and HHV7 share another common feature in that consecutive specimens remain positive for a period of weeks (Fig. 2). In contrast HHV6 DNAemia is sporadic, with a positive PBL specimen preceded and followed by negative specimens (Fig. 2). The sporadic nature of HHV6 PCR positivity in PBL has also been noted in bone marrow transplant recipients by ourselves (Appleton et al., paper submitted for publication) and others [Wilborn et al., 1994]. Studies in renal allograft recipients using culture for HHV6 revealed a similar pattern [Yoshikawa et al., 1992].

Morris et al. [1989] reported two patients with febrile illness associated with seroconversion to HHV6, one of whom also had evidence of CMV. The second patient who was seronegative for CMV throughout had a self-limiting febrile illness without exanthem, leukopenia or thrombocytopenia in association with HHV6 seroconversion. Serological cross reactions between CMV, HHV6

and HHV7 could occur, however, and make the interpretation of studies based exclusively on serology difficult. There is only one previous report on HHV7 in the immunocompromised host. Yalcin et al. [1994] studied HHV6 and HHV7 DNAemia and antibody in 16 renal transplant patients and a similar number of controls. However, their study was based on single specimens from each patient or control, collected at varying times post-transplant. Therefore, it is difficult to compare their data with our own, which were taken from regular and sequential specimens from each patient.

Although about half of organ transplant recipients have evidence of CMV DNAemia, only a minority of them develop symptomatic disease. Concurrent DNAemia with all three viruses (CMV, HHV6 and HHV7) increased the probability of clinical disease. Concurrent HHV7 and CMV DNAemia is similarly associated with increased risk of disease while the combination of HHV6 and CMV was inconclusive (Table I). In those patients with clinical symptoms of "CMV disease," only concurrent DNAemia temporally associated (within a week) with the onset of symptoms has been counted in our analysis.

Serological data for HHV6 and HHV7 (rising antibody titres) and CMV IgM results were available for the first 26 patients. A CMV IgM response was not associated with "CMV disease." Although the numbers are small, increasing antibody titres to HHV7 were associated with "CMV disease" (Fisher's exact test, $P = 0.02$). The association between rising antibody titres to HHV6 and "CMV disease" was less strong ($P = 0.07$) but also showed a trend towards statistical significance at the 95% level of confidence. Thus the serological data support the analysis based on PCR DNaemia. Those patients with "CMV disease" with rising titres to either HHV6 or HHV7 also had concurrent DNAemia to the respective virus.

The increased risk of progression to "CMV disease" in patients who are positive concurrently for both CMV and HHV7 could have many explanations. It is known that CMV by itself is immunomodulatory. Hence, CMV activity may predispose to the reactivation of HHV6 or HHV7 viruses. However we observed that HHV7 DNAemia usually preceded the CMV rather than vice versa (Fig. 2).

Alternatively, it is possible that patients who were most severely immunosuppressed are those most likely to progress to symptomatic "CMV disease," and were also the ones most likely to reactivate the other herpesviruses (providing the allograft recipient of the donor organ has latent virus within them). Thus the detection of multiple viruses may merely reflect the more severely immunocompromised patient. CMV DNAemia was more frequent in those patients receiving additional immunosuppression (intravenous steroids or anti-thymocyte globulin) as anti-rejection therapy when compared with those not receiving additional immunosuppression. Although there is an apparent increase in HHV6 and HHV7 DNAemia in patients receiving anti-rejection therapy, these differences were not statistically significant (HHV6 $P = 0.25$ and HHV7 $P = 0.46$).

All the patients with "CMV disease" had been treated for graft rejection with intravenous steroids or anti-thymocyte globulin. To investigate the potential confounding effect of anti-rejection therapy on our hypothesis, we analysed the subset of 21 patients receiving anti-rejection therapy, excluding those who did not receive additional intravenous steroids or anti-thymocyte globulin from the analysis. The association between concurrent virus DNAemia and disease is almost unchanged, suggesting that immunosuppressive anti-rejection therapy, by itself, does not explain the association observed.

HHV7 and HHV6 infect CD4 positive T lymphocytes. Infection of CD4 cells is associated with an alteration of cell surface markers (i.e. cell surface CD3; CD4; HLA-A,B,C and DR) and with dysregulation of cell function [Furukawa et al., 1994]. It is conceivable, therefore, that either HHV7 or HHV6 could act as a co-factor in precipitating clinical disease in patients who have otherwise asymptomatic CMV infection. There is a case report of lethal chickenpox following a primary HHV6 infection in an immunocompetent and otherwise healthy child [Ueda et al., 1994]. There is also speculation [Lusso and Gallo, 1994] that HHV6 may be a co-factor in progression of HIV disease, although no direct clinical evidence has been adduced to support it [Griffiths et al., 1994]. To our knowledge, there is no other evidence suggesting that either HHV6 or HHV7 can act as co-factors in any other viral disease. The data we have observed are compatible with the hypothesis that HHV7 (and possibly HHV6) may act as co-factors in potentiating disease in patients with CMV infection, although these observations require confirmation.

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